DETAILED ACTION

This action is in response to claims filed on 3/26/2010 and arguments filed 1/11/2010.

Claims 5, 9, 12-16 are pending.

The claims amendments of 3/26/2010 are technically non compliant as "an" in step e of claim 1 and "n" in claim 15 have previously been deleted from the claims but are indicated as being deleted from the instant claims. The examiner is examining the application in spite of these deficiencies but notes that further non-compliant response may result in notice of non-compliances.

The previous objection to the claims have been withdrawn in view of the amendment.

The previous 112-2nd paragraph rejections have been withdrawn in view of the amendment.

The 103 of record has been withdrawn in view of the amendment requiring the determination of phospholipidosis based on the claimed SEQ ID NO.

Claim Objections

1. Claims 5, 9, 12-16 are objected to because of the following informalities:

Claim 5 recites, "exposing each of two or more samples containing mammalian cells to each of two or more compounds known to induce phospholipidosis and exposing each of two or more samples containing mammalian cells to each of two or more compounds known not to induce phospholipidosis, wherein each sample is exposed to a single test compound." This language is convoluted and awkward. It is

suggested that clearer language be presented such as, "two or more samples of mammalian cells are each exposed to a different compound known to cause phospholipidosis and two or more samples of mammalian cells are exposed to compounds known not to induce phospholipidosis." All dependent claims are objected to as they have all the limitations from the claims from which they depend.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 5, 9, 12-16 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for

> For a method of predicting phospholipidosis induction potential in a human cells for a test compound comprising:
>
> A). treating at least ten samples of the human cells with different compounds known to induce phospholipidosis, determining the level of expression of SEQ ID NO 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23 from the samples treated with compounds known to induce phospholipidosis and ten control samples of the human cells not treated with the compounds; determining the average value of the expression variation rate for SEQ ID NO 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23 relative to the control samples
>
> B) treating a sample of the human cells with a test compound of unknown phospholipidosis inducing potential; determining the level of expression of SEQ

ID NO 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23 from the sample treated a test compound of unknown phospholipidosis inducing potential and control samples not treated with the compound of unknown phospholipidosis inducing potential; determining the average value of the expression variation rate for SEQ ID NO 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23 relative to the control samples

C) comparing the average value of expression variation of the test compound of unknown phospholipidosis inducing potential and the average value of expression vacation in part A) and predicting the test compound of unknown phospholipidosis having a average value of expression variation greater then A) is predicted to induce phospholipidosis in the human cells tested.

, does not reasonably provide enablement for determination of phospholipidosis potential based on an average value expression variation rate is not less then the standard value determined for a compound known not to induce phospholipidosis or in any non-human cells. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

There are many factors to be considered when determining whether there is sufficient evidence to support that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. These factors have been described by the court in re Wands, 8 USPQ2d 1400 (CA FC 1988). Wands states at page 1404,

"Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in the Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention and the breadth of the claims:

Claim 5 is drawn to a method for predicting a phospholipidosis induction potential of a test compound, which comprises (1) determining a standard value for the judgment of the presence or absence of a phospholipidosis induction potential of the test compound, which comprises: (a) exposing each of two or more samples containing mammalian cells to each of two or more compounds known to induce phospholipidosis and exposing each of two or more samples containing mammalian cells to each of two or more compounds known not to induce phospholipidosis, wherein each sample is exposed to a single test compound; (b) detecting expression variation of a set of genes set forth as SEQ ID NOs:l, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23, in individual samples, (c) taking a fold change of the expression amount of each gene as an expression variation rate (X) of the gene when the expression amount increased upon exposure and taking an inverse number of fold change of the expression amount of each gene as an expression variation rate (X) of the gene when the expression amount decreased upon exposure, (d) calculating an average value of the expression variation rates of the 12 genes according to the following formula and determining as an average

expression variation rate for each compound: [average expression variation rate] = mlXl+m2X2+ ... +m12X12 (ml+m2+ ... +m12=1), wherein Xi (i=1-12)is the expression variation rate of each gene, mi (i=1-12)is the weight of each gene and mix12=0.2-5; (e) determining, as the standard value, an cut-off value of average variation rate capable of correctly judging the presence or absence of a phospholipidosis induction potential of the above-mentioned compounds known to induce or not to induce phospholipidosis with the probability of not less than about 70%; and (2) predicting a phospholipidosis induction potential of the test compound, which comprises: (a) detecting expression variation of a set of genes set forth as SEQ ID NOs:l, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23, in a sample containing the mammalian cell exposed to the test compound, (b) calculating an average value of the expression variation rates of the 12 genes according to the formula shown in the step (1)(c) and determining as the average expression variation rate for the test compound, (c) comparing the average expression variation rate for the test compound with the standard value obtained by the step (1); and (d) predicting that the test compound has a phospholipidosis induction potential when the average expression variation rate for the test compound is not less than the standard value.

Thu claim 5 encompasses the determination of phospholipidosis of “any” test compound in any cell by comparison of an average expression variation rate. The claim includes predicting phospholipidosis based on any variation that is not less then the standard value, which based on the claims would include a standard value for the non-inducing compounds.

The claims also encompass any “mi “and any cell.

Claim 9 depends from claim 5 and draws the invention to wherein the step (1) further comprises examining validity of the standard value comprising the following steps: (e) detecting expression variation of a set of genes set forth as SEQ ID NOs:l, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23 in a sample containing the mammalian cells exposed to a compound known to induce or not to induce phospholipidosis other than those used in the step (1)(a), (f) comparing the average expression variation rate(s) for the compound with the standard value obtained by the step (1)(e), and (g) evaluating that the standard value is valid when the presence or absence of a phospholipidosis induction potential of the compound is correctly judged based on the standard value.

Claim 12 depends from claim 5 and draws the invention to wherein the compound known to induce phospholipidosis produces a myelin-like structure in the mammal cell.

Claim 13 depends from claim 12 and draws the invention to wherein the compound known to induce phospholipidosis is selected from the group consisting of amitriptyline, chlorcyclizine, fluoxetine, amiodarone, AY-9944, chlorpromazine, imipramine, tamoxifen, perhexiline, clozapine, sertraline, clomipramine, thioridazine, zimelidine, ketoconazole, loratadine and pentamidine.

Claim 14 depends from claim 5 and draws the invention to wherein the compound known not to induce phospholipidosis is selected from the group consisting of acetaminophen, clarithromycin, disopyramide, erythromycin, flecainide, haloperidol,

levofloxacin, ofloxacin, procainamide, quinidine, sotalol, sulfamethoxazole and sumatriptan.

Claim 15 depends from claim 5 and draws the invention to wherein mi (i= 1 -1 2)=1/12.

Claim 15 depends from claim 5 and draws the invention to wherein the phospholipidosis is induced in an organ or tissue from which the mammalian cell exposed to the test compound is derived.

The amount of direction or guidance and the Presence and absence of working examples.

The specification teaches the instant invention relates to methods of predicting toxicity of drugs (1st paragraph, page 1).

The specification teaches the cell can be any cell from a mammal (page 24, lines 23-25). The specification further teaches that a mammal includes human and non-human mammals (page 25, lines 25-29).

the "average variation rate" is defined as follows. That is, an expression amount is measured for each marker gene when mammal (cells) is and is not exposed to a test compound and when the expression amount increased upon exposure, its magnification (e.g., 2 when increased two-fold) is taken as an expression variation rate (X) of each gene, and when the expression amount decreased, an inverse number of its magnification (e.g., 2 when decreased to 1/2) is taken as an expression variation rate (X) of each gene, and an average value of the expression variation rate of the total marker genes (n genes) is defined to be an average variation rate (following formula).

Average variation rate = $m_1X_1+m_2X_2+\ldots+m_nX_n$

(m1+m2+---+mn=l)

wherein mi(i=l-n) shows the weight of each gene. While the weight is not particularly limited, it is preferably mi×n=0.2-5, for example, it is the same weight for all (mi=i/n). (page 29)

Thus the specification teaches the variation rate is determined relative to cells that have not been treated with the phospholipidosis inducing compound or compound known not to induce phosopholipidosis.

The specification teaches screening of hepatoxicity be done on hepatocytes (page 35, lines 33-top page 36).

The specification teaches, "Moreover, by accumulating the data relating to known compounds by evaluation by the present method and microscopic observation, extremely accurate prediction of compound groups unknown as to the presence or absence of a PLsis induction potential becomes possible" (page 35, lines 23-27). Thus the specification suggests the gene analysis method requires an additional step of microscopic for extremely accurate prediction of PLsis.

The specification in example 1 teaches electromicroscopic analysis of 30 compounds on HepG2 cells (pages 37-40).

The specification teaches in example 2 gene expression variation of PLsis inducing genes in HEPG2 cells. The specification teaches HEPG2 cells were exposed to 17 PLsis inducing compounds and 14 PLsis non-inducing compounds for 24 hours (page 41, lines 5-10). The specification teaches the cells were frozen and RNA was analyzed. The specification teaches primers and probes were designed based on the sequences of SEQ ID NO 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21. It is noted the specification provides no data or suggestion on how these nucleic acid sequences were chosen as markers.

The specification teaches, "For each test compound, the expression variation rate of 12 genes each relative to the control group was determined. The results are shown in Table 2. In all the 12 genes examined, there was a tendency toward variation in their expression due to the exposure to a PLsis-inducing compound, and substantially no variation in their expression due to the exposure to a PLsis non-inducing compound. Therefore, it has been clarified that these 12 genes are marker genes useful for the prediction of a PLsis induction potential of a drug." Thus the specification teaches exposure of cells to "each test compound" resulted in an expression variation in the cells studied. The specification teaches all known PLsis inducing compounds resulted in an average 1.5 variation rate and 12 of 13 non-PLsis inducing compounds resulted in a variation rate of less than 1.5 (page 46, lines 10-17). Example 2 teaches addition of single compounds to cells and analysis of the cells after single treatment, which does not demonstrate predictability of treating at a cell with multiple compounds at once or sequentially and examining gene variation as claimed in claims7-9.

The specification in example 3 confirms the findings of example 2 by use of 26 compounds for determination of presence or absence of PLsis induction potential.

Presence and absence of working examples

The specification provides no examples in which PLsis was determined without the use of control samples not treated with a PLsis inducing compound or by comparison to a standard value derived from cells treated with a non-PLsis inducing compounds.

The state of prior art and the predictability or unpredictability of the art:

Post-filing art of Nioi et al (Toxilogical Sciences (2007) volume 99, pages 162-173) presents a study to validate a study of instant inventors and specification. Nioi et al reiterates that even after filing it is established that different drugs affect different cells differently , "It is important to note that both erythromycin and quinidine are known to cause PLD in vivo and in vitro in other cell types" (page 164, 1st column, last paragraph). Nioi teaches that 5 of the 8 drugs known to cause PLsis had predictability index of greater than 1.5, which is the measure the instant specification uses. However, Nioi teaches that 3 of the 8 (but amiodarone, loratadine, and tamoxifen) all had scores < 1.5 (page 165, 1st column, 1st full paragraph). Nioi et al continues, "Our results are consistent with the previously published data with a couple of notable differences (Table 4). Sawada et al. (2005) identified amiodarone, loratadine, and tamoxifen as PLD-inducing drugs with PI scores of 1.61, 1.59, and 2.15, respectively, whereas in our study all of these compounds had PI scores < 1.5 (Table 4). Consistent with our data, Atienzar et al. (2006) also generated PI scores of < 1.5 for amiodarone and tamoxifen; they did not examine loratadine (Table 4). The only other major difference in the three data sets was with fluoxetine which had a PI score consistent with it inducing PLD in our study (1.99) and in Sawada's study (3.29) but not in Atienzar's experiment (1.27) (Table 4)" (page 165, bottom 1st column to top of 2nd column). Nioi teaches that increasing doses of PLsis inducing compounds led to increase PI and thus correct prediction. Nioi teaches, "We found that only five of eight of the positive control compounds were correctly identified when a single concentration of drug was used, and these findings were consistent between experiments" (page

170). Nioi suggests that while gene expression analysis is useful, the use of multiple drug concentration improves the accuracy (predictability) of the method (page 172, 1st column, last paragraph).

The art of Cheung et al (Nature Genetics, 2003, volume 33, pages 422-425) teaches that there is natural variation in gene expression among different individuals. The reference teaches an assessment of natural variation of gene expression in lymphoblastoid cells in humans, and analyzes the variation of expression data among individuals and within individuals (replicates) p.422, last paragraph; Fig 1). The data indicates that, for example, expression of ACTG2 in 35 individuals varied by a factor of 17; and that in expression of the 40 genes with the highest variance ratios, the highest and lowest values differed by a factor of 2.4 or greater (Fig 3).

Benner et al (Trends in Genetics (2001) volume 17, pages 414-418) teaches that, "Here, the 'homology-implies-equivalency' assumption is restricted to a subset of homologs that diverged in the most-recent common ancestor of the species sharing the homologs. This strategy is useful, of course. But it is likely to be far less general than is widely thought. Two species living in the same space, almost by axiom, cannot have identical strategies for survival. This, in turn, implies that two orthologous proteins might not contribute to fitness in exactly the same way in two species" (see page 414, 3rd column last full paragraph). Benner specifically describes that although the leptin gene homologs have been found in mice and humans, their affect is different (see page 414, 3rd column last paragraph-3rd column page 415). Benner specifically teaches that the leptin gene in mice plays a major role in obesity, but no such effect has been

demonstrated in humans due perhaps to the different evolutionary forces. Benner thus teaches that the activity and function of genes in different species is unpredictable.

The level of skill in the art:

The level of skill in the art is deemed to be high.

Quantity of experimentation necessary:

In order to practice the invention as claimed, one would first have to establish that a predicative relationship exists between the rate of variation of expression of SEQ ID NO 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 in cells treated with a known phospholipidosis inducing compound or a compound known not to induce phospholipidosis. This would be unpredictable as the specification teaches the rate of variation was determined relative to untreated cells, while the claims merely require cells treated with compounds that induce phospholipidosis or compounds that do not induce phospholipidosis. Thus due to the unpredictability of gene expression as taught by Cheung and the lack of specific guidance of the claimed method the instant claims are unpredictable.

Further it would be unpredictable as the claims appear to allow comparison either a rate of variation from the compounds known not to indicate phospholipidosis or compounds known not to induce phospholipidosis. It would thus be unpredictable to determine a compound induces phospholipidosis based on it having a variation rate of not less then the variation rate of compounds known not to induce phospholipidosis.

It would further be unpredictable to use only two samples treated with inducing compounds for comparison based on the unpredictability of gene expression. If both of

the samples treated with phospholipidosis inducing compounds had decreased expression due to natural variability it would result in all compounds being indicated as phospholipidosis inducing compounds, while the inclusion of 10 or more samples minimizes the unpredictability.

It would further be unpredictable to predict phosphlipidosis in non-human cells by the detection of human genes. It would be unpredictable as the claimed SEQ ID NO are to human genes and other mammals would not predictably have the same SEQ ID NO. Further it would be unpredictable as Benner teaches gene homologs from different species often have different functions due to different evolutionary pressures.

Further it would be unpredictable to practice the invention in a tissue or organ as claim 16 appears to require as this would involve whole animals studies which do not appear to be envisioned or enabled by the instant specification or prior art of record.

Further it would be unpredictable to practice the invention as claimed with mi being variable. The specification teaches the mi=1/12 works, however the independent claim encompasses every mi being different and thus is unpredictable.

Therefore, in light of the breadth of the claims, the lack of guidance in the specification, the high level of unpredictability in the associated technology, the nature of the invention, the negative teachings in the art, and the quantity of unpredictable experimentation necessary to practice the claimed invention, it would require undue experimentation to practice the invention as claimed.

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 5, 9, 12-16 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 recites the limitation "wherein in each sample is exposed to a single test compound" in the last line of step a. It is unclear which samples are being referred to as well as if “a single test compound” refers to compounds known to induce phospholipidosis and compounds known not to induce phospholipidosis. Claims 9 and 12-16 are rejected as they depend from claim 5.

Claim 5 recites the limitation "the 12 genes" in first line of step b. There is insufficient antecedent basis for this limitation in the claim. This aspect of the rejection can easily be overcome by amending the claims to recite, “the set of genes.”

Claim 16 recites, “wherein the phospholipidosis is induced in an organ or tissue from which the mammalian cell exposed to the test compound is derived." It is unclear if the claim is attempting to require phospholipidosis is tested in organs or tissues or if the claim is trying to extrapolate that phospholipidosis likely will occur in the tissue or organ from which the sample was obtained.

Summary

No claims are allowed.

Conclusion

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP

§ 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN C. POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-4:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634